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Saha N; Schwer B; Shuman S

10021, USA.

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L7
     ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS
     2000:589929 CAPLUS
AN
DN
     133:172151
ΤI
     Screening for inhibitors of mRNA cap formation for use as antimycotics
     using host cells with fungal or mammalian capping enzymology
IN
     Shuman, Stewart
PA
     USA
SO
     U.S., 62 pp.
     CODEN: USXXAM
DT
     Patent
     English
LA
FAN.CNT 3
     PATENT NO.
                    KIND DATE
                                          APPLICATION NO. DATE
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PΙ
     US 6107040
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                            20000822
                                          US 1998-188579
                                                           19981109
     US 6232070
                                           US 1999-315444
                      В1
                            20010515
                                                            19990520
     WO 2000063433
                      A1
                            20001026
                                          WO 1999-US26520 19991109
         W: AU, CA, JP, MX
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
     EP 1127164
                      A1
                            20010829
                                         EP 1999-962733
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            AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
PRAI US 1998-188579
                            19981109
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     US 1999-315444
                      Α
                            19990520
     WO 1999-US26520
                      W
                            19991109
     This invention provides methods for the discovery of mols. that target an
AΒ
     essential aspect of eukaryotic gene expression-the formation of
     the mRNA 5' cap m7GpppN. An underlying
     principle of this invention is the use of a different strains of a test
     organism that differ only in the compn. or source of the essential
     cap-forming enzymes. The invention provides isogenic yeast
     strains that derive all their capping activities from
     fungal sources vs. mammalian sources. These strains form the
     basis of a differential growth inhibition assay to identify mols. that
     specifically target the fungal capping app. This
     invention also provides a method to screen in vitro for mols. that
inhibit
     fungal RNA triphosphatase, an essential enzyme that catalyzes the
     first of three steps in cap synthesis. The ability of human
     capping activities to replace those of Saccharomyces
     cerevisiae is demonstrated.
RE.CNT 2
(1) Ho; Molecular and Cellular Biology 1998, V18(9), P5189 CAPLUS
(2) Yue; Mammalian capping enzyme complements mutant Saccharomyces cerevisiae
    lacking mRNA guanylytransferase and selectively binds the elongating form
    of RNA polymerase 2 1997, V94, P12898 CAPLUS
L7
    ANSWER 4 OF 4
                      MEDLINE
                                                       DUPLICATE 2
ΑN
     1999278431
                   MEDLINE
DN
     99278431 PubMed ID: 10347220
ΤI
     Characterization of human, Schizosaccharomyces pombe, and Candida
albicans
     mRNA cap methyltransferases and complete replacement of the yeast capping
     apparatus by mammalian enzymes.
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Molecular Biology Program, Sloan-Kettering Institute, New York, New York

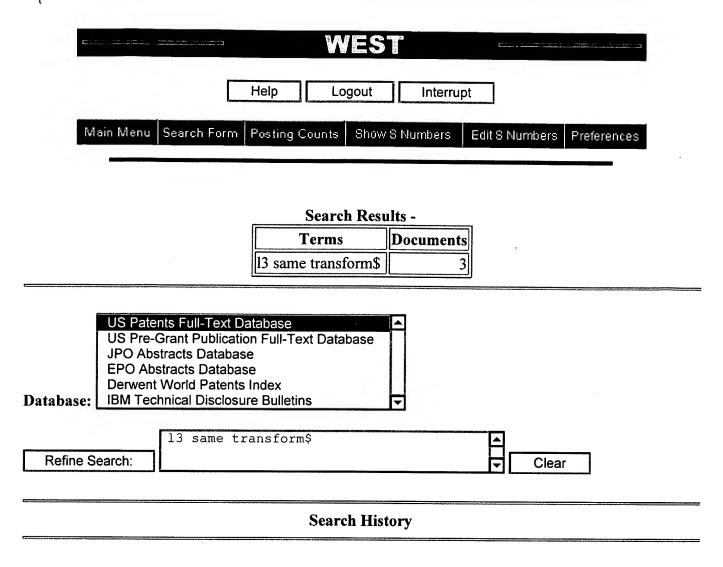
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NC GM52470 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 4) 274 (23) 16553-62.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
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- CY United States
- DT Journal; Article; (JOURNAL ARTICLE)
- LA English
- FS Priority Journals
- EM 199907
- ED Entered STN: 19990714

Last Updated on STN: 19990714 Entered Medline: 19990701

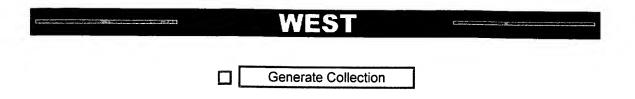
AB Human and fission yeast cDNAs encoding mRNA (guanine-N7) methyltransferase were identified based on similarity of the human (Hcmlp;

476 amino acids) and Schizosaccharomyces pombe (Pcmlp; 389 amino acids) polypeptides to the cap methyltransferase of Saccharomyces cerevisiae (Abdlp). Expression of PCM1 or HCM1 in S. cerevisiae complemented the lethal phenotype resulting from deletion of the ABD1 gene, as did expression of the NH2-terminal deletion mutants PCM1(94-389) and HCM1(121-476). The CCM1 gene encoding Candida albicans cap methyltransferase (Ccmlp; 474 amino acids) was isolated from a C. albicans genomic library by selection for complementation of the conditional growth phenotype of S. cerevisiae abdl-ts mutants. Human cap methyltransferase was expressed in bacteria, purified, and characterized. Recombinant Hcmlp catalyzed quantitative S-adenosylmethionine-dependent conversion of GpppAcapped poly(A) to m7GpppA-capped poly(A). We identified by alanine-scanning mutagenesis eight amino acids (Asp-203, Gly-207, Asp-211, Asp-227, Arg-239, Tyr-289, Phe-291, and Phe-354) that are essential for human cap methyltransferase function in vivo. All eight residues are conserved in other cellular cap methyltransferases. Five of the mutant human proteins (D203A, R239A, Y289A, F291A, and F354A) were expressed in bacteria and found to be defective in cap methylation in vitro. Concordance of mutational effects on Hcmlp, Abdlp, and vaccinia capping enzyme underscores a conserved structural basis for cap methylation in DNA viruses, yeast, and metazoans. This is in contrast to the structural and mechanistic divergence of the RNA triphosphatase components of the yeast and metazoan capping systems. Nevertheless, we demonstrate that the entire three-component yeast capping apparatus, consisting of RNA 5'-triphosphatase (Cetlp), RNA guanylyltransferase (Ceglp), and Abdlp could be replaced in vivo by the two-component mammalian apparatus consisting of a bifunctional triphosphatase-guanylyltransferase Mcelp and the methyltransferase Hcm1(121-476)p. Isogenic yeast strains with fungal versus mammalian capping systems should facilitate rational screens for antifungal drugs that target cap formation in vivo.



Today's Date: 9/6/2001

DB Name	Query	Hit Count	Set Name
USPT	13 same transform\$	3	<u>L4</u>
USPT	12 same 5	36	<u>L3</u>
USPT	11 same mRNA same cap\$	92	<u>L2</u>
USPT	gene same replac\$	8727	<u>L1</u>



L3: Entry 7 of 36

File: USPT

Jan 16, 2001

DOCUMENT-IDENTIFIER: US 6174669 B1

TITLE: Method for making full-length cDNA libraries

BSPR:

As conventional methods for synthesizing full-length cDNAs, the following methods can be mentioned; the method utilizing a Cap binding protein of yeast or Hela cells for labeling the 5' Cap site (I. Edery et al., "An Efficient Strategy To Isolate Full-length cDNAs Based on an MRNA Cap Retention Procedure (CAPture)", MCB, 15, 3363-3371, 1995); the method where phosphates of incomplete cDNAs without 5' Cap are removed by using alkaline phosphatase and then the whole cDNAs are treated with de-capping enzyme of tobacco mosaic virus so that only the full-length cDNAs have phosphates (K. Maruyama et al., "Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides", Gene, 138, 171-174, 1995., S. Kato et al., "Construction of a human full-length cDNA bank", Gene, 150, 243-250, 1995) and the like.